

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (*Previously Presented*) A method for the preparation of a polypeptide of interest in authentic form, said method comprising the steps of:

(i) providing a fusion protein comprising, from its N-terminal to its C-terminal, (a) a fusion partner, (b) a Granzyme B protease recognition site comprising a Granzyme B protease cleavage site, (c) a polypeptide of interest, wherein said cleavage site is adjacent to the polypeptide of interest, and

(ii) contacting said fusion protein with Granzyme B protease to cleave it at said cleavage site to yield said polypeptide of interest in authentic form.

2. (*Currently Amended*) A method according to claim 1, wherein the Granzyme B protease recognition site [[has]] comprises an amino acid sequence of the general formula:

P4 P3 P2 P1↓

wherein

P4 is amino acid I or V,

P3 is amino acid E, Q or M,

P2 is X, where X denotes any amino acid,

P1 is amino acid D, and

↓ is said Granzyme B protease cleavage site.

3. (*Previously Presented*) A method according to claim 1, wherein the Granzyme B protease recognition site has an amino acid sequence selected from the group consisting of ICPD↓, IEAD↓, IEPD↓, IETD↓, IQAD↓, ISAD↓, ISSD↓, ITPD↓, VAPD↓, VATD↓, VCTD↓, VDPD↓, VDSD↓, VEKD↓, VEQD↓, VGPD↓, VEID↓, VRPD↓, VTPD↓, LEED↓, LEID↓, LGND↓, LGPD↓, and AQPD↓, and wherein ↓ is said Granzyme B protease cleavage site.

4. (*Original*) A method according to claim 2, wherein the general formula furthermore comprises the amino acids P1' and P2' resulting in the general formula

P4 P3 P2 P1↓P1'P2', wherein P1' is X where X denotes any amino acid, P2' is G, and wherein P1' and P2' is a part of the polypeptide of interest.

5. *(Original)* A method according to claim 2, wherein the general formula furthermore comprises the amino acids P1', P2', P3' and P4' resulting in the general formula P4 P3 P2 P1↓P1'P2'P3'P4', wherein P4' is D or E, and wherein P1', P2', P3' and P4' is a part of the polypeptide of interest.

6. *(Original)* A method according to claim 1, wherein the polypeptide of interest is selected from the group consisting of an enzyme, a polypeptide hormone, a single chain antibody variable region fragment, and apolipoprotein A.

7. *(Original)* A method according to claim 6, wherein the polypeptide hormone is selected from the group consisting of somatotrophin, glucagon, insulin and interferon.

8. *(Original)* A method according to claim 6, wherein the enzyme is Granzyme B.

9. *(Original)* A method according to claim 1, wherein the fusion partner is an affinity-tag.

10. *(Original)* A method according to claim 9, wherein the affinity-tag is selected from the group consisting of a polyhistidine-tag, a polyarginine-tag, a FLAG-tag, a Strep-tag, a c-myc-tag, a S-tag, a calmodulin-binding peptide, a cellulose-binding peptide, a chitin-binding domain, a glutathione S-transferase-tag, and a maltose binding protein.

11. *(Original)* A method according to claim 1, wherein the Granzyme B protease is selected from the group consisting of human Granzyme B protease, mouse Granzyme B protease and rat Granzyme B protease.

12. *(Original)* A method according to claim 11, wherein the Granzyme B protease is a human Granzyme B protease variant as shown in SEQ ID NO 57, wherein the Cysteine residue no. 228 (chymotrypsinogen numbering) is mutated to Phenylalanine.

13. *(Original)* A method according to claim 1, wherein the Granzyme B protease is in an immobilised form.

14. *(Original)* A method according to claim 13, wherein the Granzyme B protease is immobilised via the C-terminus.

15. *(Original)* A method according to claim 13, wherein the Granzyme B protease is immobilised via a lysine amino acid residue.

16. *(Previously Presented)* A method according to claim 10, wherein the affinity-tag is a polyhistidine-tag, and wherein the fusion protein is contacted with said Granzyme B protease in the presence of Ni^{2+} ions and Nitrilotriacetic Acid (NTA).

17. *(Original)* A method according to claim 15, wherein the concentration of Ni^{2+} is in the range of 1-20 mM, and the concentration of NTA is in the range of 1-20 mM.

18. *(Previously Presented)* A fusion protein comprising, from its N-terminal to its C-terminal, (a) a fusion partner, (b) a Granzyme B protease recognition site comprising a Granzyme B protease cleavage site, and (c) a polypeptide of interest, wherein said cleavage site is adjacent to the polypeptide of interest.

19. *(Currently Amended)* A fusion protein according to claim 18, wherein the Granzyme B protease recognition site [[has]] comprises an amino acid sequence of the general formula:

P4 P3 P2 P1↓

wherein

P4 is amino acid I or V₁

P3 is amino acid E, Q or M₁

P2 is X, where X denotes any amino acid,

P1 is amino acid D, and

↓ is said Granzyme B protease cleavage site.

20. *(Previously Presented)* A fusion protein according to claim 18, wherein the Granzyme B protease recognition site has an amino acid sequence selected from the group consisting of ICPD↓, IEAD↓, IEPD↓, IETD↓, IQAD↓, ISAD↓, ISSD↓, ITPD↓, VAPD↓, VATD↓, VCTD↓, VDPD↓, VDSD↓, VEKD↓, VEQD↓, VYPD↓, VEID↓, VRPD↓, VTPD↓, LEED↓, LEID↓, LGND↓, LGPD↓, and AQPD↓, and wherein ↓ is said Granzyme B protease cleavage site.

21. *(Original)* A fusion protein according to claim 19, wherein the general formula furthermore comprises the amino acids P1' and P2' resulting in the general formula P4 P3 P2

$P1 \downarrow P1'P2'$, wherein $P1'$ is X where X denotes any amino acid, $P2'$ is G, and wherein $P1'$ and $P2'$ is a part of the polypeptide of interest.

22. *(Original)* A fusion protein according to claim 19, wherein the general formula furthermore comprises the amino acids $P1'$, $P2'$, $P3'$ and $P4'$ resulting in the general formula $P4 P3 P2 P1 \downarrow P1'P2'P3'P4'$, wherein $P4'$ is D or E, and wherein $P1'$, $P2'$, $P3'$ and $P4'$ is a part of the polypeptide of interest.

23. *(Original)* A fusion protein according to claim 18, wherein the polypeptide of interest is selected from the group consisting of an enzyme, a polypeptide hormone, a single chain antibody variable region fragment, and apolipoprotein A.

24. *(Original)* A fusion protein according to claim 23, wherein the polypeptide hormone is selected from the group consisting of somatotrophin, glucagon, insulin and interferon.

25. *(Original)* A fusion protein according to claim 23, wherein the enzyme is Granzyme B.

26. *(Original)* A fusion protein according to claim 25, wherein Granzyme B comprises a C-terminal polyhistidine-tag.

27. *(Original)* A fusion protein according to claim 25, selected from the group consisting of pro-IEPD-GrB-H6 (SEQ ID NO 2) and pro-IEAD-GrB-H6 (SEQ ID NO 3).

28. *(Original)* A fusion protein according to claim 25, selected from the group consisting of pro-IEPD-GrB-H6 C228A (SEQ ID NO 5), pro-IEPD-GrB-H6 C228T (SEQ ID NO 6), pro-IEPD-GrB-H6 C228V (SEQ ID NO 7), and pro-IEPD-GrB-H6 C228F (SEQ ID NO 8).

29. *(Original)* A fusion protein according to claim 25, wherein the enzyme Granzyme B is a human Granzyme B protease variant wherein the Cystein residue no. 228 (chymotrypsinogen numbering) is mutated to Phenylalanine.

30. *(Original)* A fusion protein according to claim 25, wherein the human Granzyme B protease variant is as shown in SEQ ID NO 57.

31. *(Original)* A fusion protein according to claim 18, wherein the fusion partner is an affinity-tag.

32. *(Original)* A fusion protein according to claim 31, wherein the affinity-tag is selected from the group consisting of a polyhistidine-tag, a polyarginine-tag, a FLAG-tag, a Strep-tag, a c-myc-tag, a S-tag, a calmodulin-binding peptide, a cellulose-binding peptide, a chitin-binding domain, a glutathione S-transferase-tag, and a maltose binding protein.

33. *(Original)* A human Granzyme B protease variant wherein the Cystein residue no. 228 (chymotrypsinogen numbering) is mutated to Phenylalanine.

34. *(Original)* A human Granzyme B protease variant according to claim 33, as shown in SEQ ID NO 57.

35. *(Previously Presented)* A method of cleaving a fusion protein comprising contacting said fusion protein with the human Granzyme B protease variant according to claim 33.

36. *(Previously Presented)* An isolated nucleic acid sequence encoding the fusion protein according to claim 19 or the human Granzyme B protease variant according to claim 33.

37. *(Original)* A recombinant vector comprising the isolated nucleic acid sequence according to claim 36.

38. *(Original)* A host cell transformed with a vector according to claim 37.

39. *(Previously Presented)* A method for the production of a fusion protein according to claim 18 or a human Granzyme B protease variant according to claim 33, comprising the steps of:

- (i) providing a recombinant vector comprising the isolated nucleic acid sequence according to claim 36 operatively linked to a promotor,
- (ii) transforming a host cell with said recombinant vector,
- (iii) culturing said host cell under conditions to express said fusion protein or human Granzyme B protease variant, and
- (iv) optionally isolating said fusion protein or human Granzyme B protease variant.